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MDMA induced dopamine release in vivo: role of endogenous serotonin

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Summary. Acting as a substrate at the serotonin (5-HT) transporter, (+)-MDMA (3,4-methylenedioxymethamphetamine), is a potent releaser of 5-HT and causes toxicity to 5-HT neurons after repeated exposure. (+)-MDMA also releases dopamine (DA), although with less potency. Since we have shown previously that the intrastriatal application of 5-HT facilitates DA release, it was hypothesized that increased release of striatal 5-HT after MDMA may influence extracellular levels of DA. Using microdialysis in vivo, we found that (+)-MDMA (4.7 μmol/kg, i.v.) administration increased extracellular striatal DA levels to 501% of control ($p < 0.01$, $n = 12$). However, in the presence of fluoxetine (14.4 μmol/kg, s.c.), which prevents (+)-MDMA effects on 5-HT release, the (+)-MDMA-induced increase in DA was significantly less (to 375% of control, $p < 0.05$, vs. no fluoxetine, $n = 8$). In vitro studies with striatal slices, to test drug selectivity, showed that (+)-MDMA (0.3–3 μM) increased extracellular levels of both DA and 5-HT in a dose-dependent manner. Fluoxetine (3 μM) completely blocked the effects of (+)-MDMA on 5-HT release, but did not alter (+)-MDMA-induced DA release in vitro. The selective DA transport inhibitor GBR-12909 (1 μM), blocked (+)-MDMA's effect on DA release. It is concluded that 5-HT release after (+)-MDMA treatment partially contributes to (+)-MDMA's effect on DA release in vivo.

Keywords: Dopamine serotonin, microdialysis, striatum, MDMA, brain slices

Abbreviations

MDMA 3,4-methylenedioxymethamphetamine, *DA* dopamine, *5-HT* serotonin, *TPH* tryptophan hydroxylase, *DAT* dopamine transporter, *SERT* serotonin transporter.

Introduction

MDMA (3,4-methylenedioxymethamphetamine, "Ecstasy") is a widely abused psychomotor stimulant with behavioral effects related to both amphetamines and hallucinogens (Peroutka, 1987; Peroutka et al., 1988). On a biochemical level, MDMA promotes serotonin (5-HT) release via an action at the 5HT transporter (Rudnick and Wall, 1992) and after repeated exposure, MDMA is neurotoxic to 5-HT neurons (Mc Kenna and Peroutka, 1990; Battaglia et al., 1991). The acute effects of MDMA, such as decreases in 5-HT tissue levels and tryptophan hydroxylase activity (Stone et al., 1987; Schmidt and Taylor, 1988), appear to be reversible, however the long-term toxicity of MDMA is characterized by an irreversible loss of 5-HT neuronal markers, including a decrease in 5-HT transporters (Stone et al., 1987; McKenna and Peroutka, 1990) and axon terminals (Molliver et al., 1990; Battaglia et al., 1991). Extensive and irreversible serotonergic neurotoxicity has been demonstrated in laboratory animals, with monkeys being more sensitive than rats to MDMA-induced 5-HT depletion (Ricaurte, 1989; Ricaurte et al., 1992).

Several studies, including the present one, have shown that MDMA also releases dopamine (DA), (McKenna and Peroutka, 1990; Yamamoto and Spanos, 1988; Nash and Yamamoto, 1992) although with less efficacy in vitro when compared to its effect on 5-HT. Perhaps more importantly, DA has been shown to be a required intermediary in MDMA-induced toxicity to the 5-HT system. This conclusion is based on the observations that pretreatment with either reserpine, α-methyl-p-tyrosine (Brodkin et al., 1993), 6-hydroxydopamine (Stone et al., 1988), or the selective DA transport inhibitor GBR 12909 (Stone et al., 1988) prevent MDMA toxicity to 5-HT neurons. The protective effect of DA depletion is reversible by l-dopa administration, further supporting a role for DA in mediating the effects of MDMA on 5HT containing neurons (Schmidt et al., 1990b).

Activation of $5HT_2$ receptors may also contribute to MDMA-induced neurotoxicity since administration of $5\text{-}HT_2$ receptor agonists potentiated DA synthesis after MDMA administration (Ugedo et al., 1989; Schmidt et al., 1992; Huang and Nichols, 1993). Moreover, pretreatment with $5\text{-}HT_2$ antagonists prevented MDMA toxicity (Schmidt et al., 1990a,b) although the precise mechanism of $5\text{-}HT_2$ antagonism remains to be elucidated.

Since our previous results (Benloucif and Galloway, 1991; Benloucif et al., 1993; Galloway et al., 1993; West and Galloway, 1996), as well as others (Chen et al., 1992; Parsons and Justice, 1993; Yadid et al., 1994), suggest that the intracerebral application of 5-HT facilitates DA release via an axo-axonic mechanism, we hypothesized that increased extracellular 5-HT after MDMA contributed to the MDMA effect on extracellular DA in vivo. In order to explore further the mechanism of (+)-MDMA on 5-HT and DA release, the effects of the selective 5-HT transporter blocker fluoxetine, and GBR-12909, a selective DA transporter blocker, were investigated with respect to their role in the action of (+)-MDMA. Additionally, the effect of the $5\text{-}HT_2$ antagonist, ketanserin, on (+)-MDMA-induced 5-HT release in vitro is

reported. The results confirm that MDMA acts at both 5HT and DA transporters and that MDMA's effect on striatal DA function in vivo is partially dependent on endogenous 5HT.

Materials and methods

Microdialysis (in vivo)

Male Harlan Sprague-Dawley rats were kept on a 12:12 hour light/dark cycle with food and water ad libitum. Rats weighing between 275–300 g were anesthetized with 400 mg/kg (i.p.) of chloral hydrate (160 g/L) and a tail vein cannula inserted to administer maintenance doses of anesthetic (16 mg/30 min). Concentric dialysis probes [4 mm exposed membrane, modified design of Robinson and Whishaw (1988), Benloucif and Galloway (1991)] were implanted bilaterally into the anterior striatum (+0.7 mm anterior, ±3.0 mm lateral, −7.0 mm ventral to bregma; Paxinos and Watson, 1986). Body temperature was monitored via a rectal probe and maintained at 37°C using a heating pad. The dialysis probes were constantly perfused (2 μl/min) with artificial cerebral spinal fluid (147 mM NaCl, 3 mM KCl, 0.8 mM $MgSO_4$, 1.2 mM $CaCl_2$, 2 mM NaH_2PO_4, 2 mM Na_2HPO_4, pH 7.3) using a CMA microperfusion pump. Every 20 minutes 40 μl samples were collected into 10 μl HPLC mobile phase (containing 12% MeOH, 0.1 M monobasic sodium phosphate buffer, 2 mM octylsulfonic acid and 0.1 mM EDTA, pH 4.5) to prevent neurotransmitter degradation.

Fluoxetine (14.4 μmol/kg, Eli Lilly, Indianapolis, IN) was dissolved in saline and administered s.c. in a volume of 1 ml/kg 1 hr before (+)-MDMA. (+)-MDMA (4.7 μmol/kg, National Institute on Drug Abuse (NIDA)) was dissolved in saline and injected i.v. (1 × body weight). Extracellular DA levels increased within 10 min after (+)-MDMA administration with a peak effect in the fraction immediately following drug delivery (Fig. 1). Drugs were administered after a stable DA baseline was established. The (+)-stereoisomer of MDMA was utilized in the present study since it is a more potent 5-HT releasing agent than the (−)-stereoisomer (Schmidt et al., 1986; Schmidt, 1986; Hiramatsu and Cho, 1990; Galloway et al., unpublished observations).

Levels of extracellular DA were immediately analyzed by electrochemical detection with high performance liquid chromatography (HPLC-EC). For the HPLC detection a reverse phase column (BAS, 5 micron, C-18, flow rate 0.8 ml/min) was used and the applied potential was 0.7 V vs. a Ag/AgCl reference electrode (Galloway et al., 1986). Three consecutive stable fractions of basal DA levels were defined as control and drug effects were assessed as percent change over control. The data were analyzed by ANOVA and Bonferroni t-test, as indicated in the figure legend. Following the experiment, rats were decapitated, brains were removed and stored in formalin sucrose for probe placement verification.

Slice experiments (in vitro)

Striata from male Harlan Sprague-Dawley rat brains were rapidly dissected onto an iced plate and slices (0.3 × 0.3 mm) prepared with a McIlwain tissue chopper (Clark et al., 1991). Slices were pooled and placed into 19 ml oxygenated (95% O_2, 5% CO_2) Krebs Ringer Mops buffer (containing 128 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 11.1 mM glucose, 15.8 mM MOPS, 1.2 mM $MgSO_4$, 10 μM l-tryptophan, and 10 μM l-tyrosine, adjusted to pH 7.4). Following a 5 minute preincubation period at 37°C, slices were washed and resuspended in fresh buffer. Aliquots were then placed into incubation tubes and equilibrated in a shaking water bath at 37°C. Fluoxetine (3 μM) or ketanserin (1 μM, Janssen Pharmaceuticals) were added 10 minutes prior to (+)-MDMA (0.3–3 μM, NIDA), GBR-12909 (1 μM, RBI, Natick, MA) was added 5 minutes prior to (+)-MDMA. All drugs were dissolved in buffer. Fifteen minutes following (+)-MDMA addition, an aliquot of tissue-free buffer was acidified with 0.21 N $HClO_4$. Levels of either 5-HT or DA

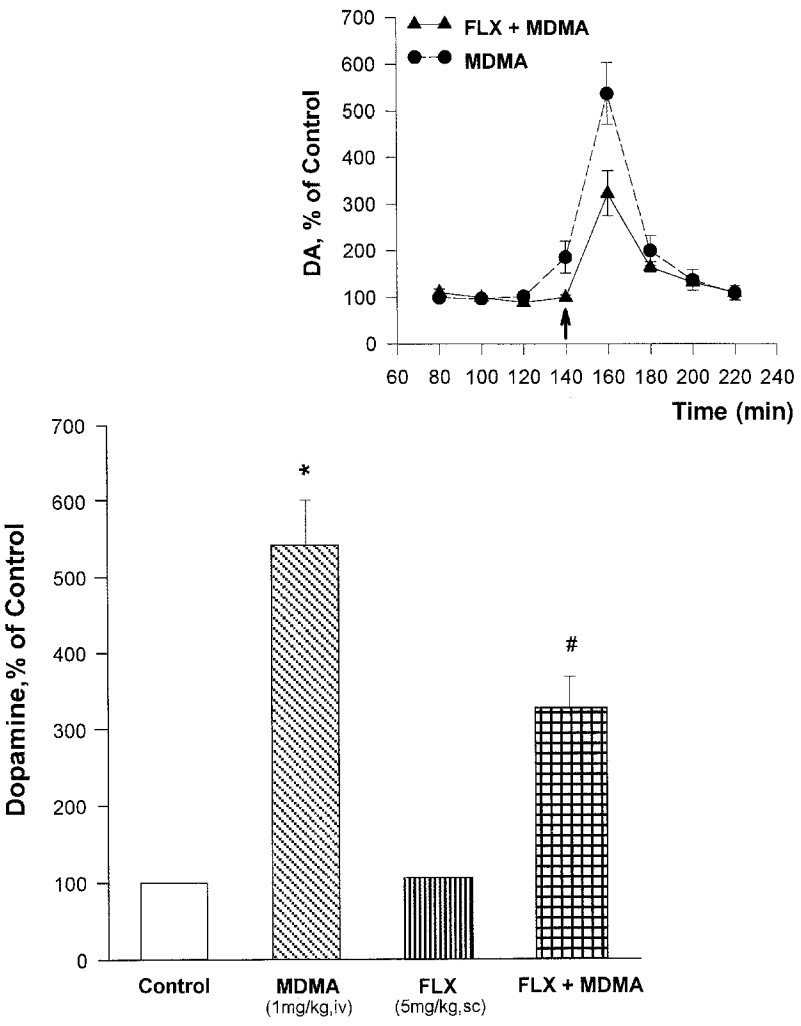

Fig. 1. Fluoxetine reduced MDMA-induced DA release in vivo. Under control conditions (+)-MDMA (4.7 μmol/kg, i.v.) increased extracellular DA to 501 ± 66% of basal levels. The arrow indicates the time of MDMA administration. Pretreatment with fluoxetine (14.4 μmol/kg, s.c.) significantly reduced the effect of MDMA on DA to 375 ± 48% of basal levels. (*FLX* fluoxetine; * = $p < 0.01$ vs. respective control, n = 12; # = $p < 0.05$ vs. MDMA alone, n = 8)

(determined by HPLC-EC) in this sample were defined as extracellular DA or 5-HT, shown in Figs. 2–4. Extracellular 5-HT or DA levels were expressed as nM per mg protein for each incubate. The remaining tissue was acidified with 2.1 N $HClO_4$, sonically disrupted, and aliquots obtained for protein analysis by the Bradford method. Under these conditions, (+)-MDMA induced release of either DA or 5HT is assumed to be via an action at the transporter since the effect is Ca^{++}-independent, Na^{+}-dependent, stereoselective (Galloway et al., unpublished observations), and blocked by inhibitors specific for each transporter (see Results).

Data from the dose response experiments were transformed to % control and presented graphically as a smooth line through the data points using the cubic spline method (Sigma Plot, Jandel Scientific). Statistical analysis was performed using a two-way analysis of variance and Dunnett's t-test.

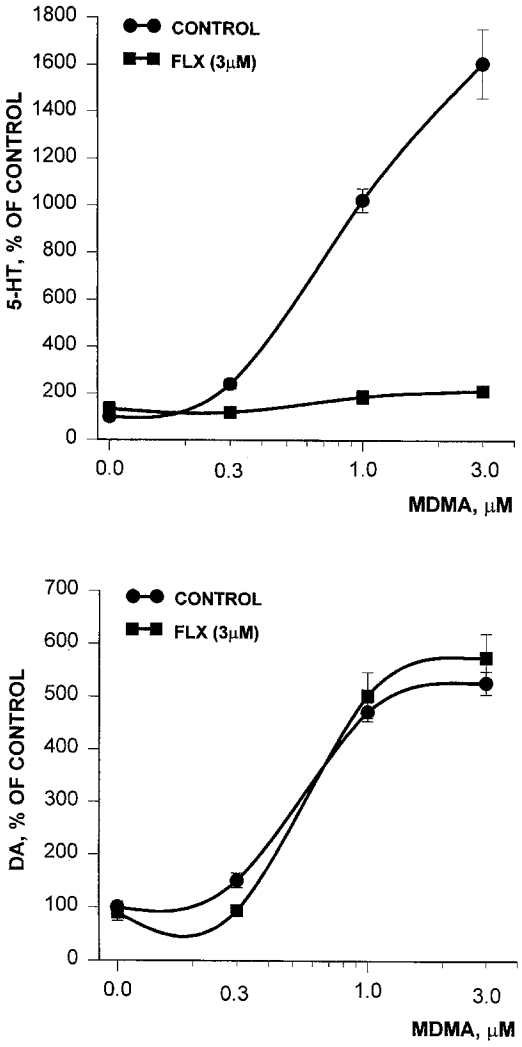

Fig. 2. *Top:* MDMA (•, 0.3–3 μM) increased extracellular 5-HT in a dose-dependent manner, an effect blocked by preincubation with fluoxetine (■, 3 μM). *Bottom:* MDMA (•) increased extracellular DA in a dose-dependent manner, however, fluoxetine (■) did not alter the effect of MDMA. Each data point represents the mean ± S.E.M. of n = 4 replicates. If no error bar is shown, its size did not exceed that of the data point symbol. Two-way ANOVA revealed a significant ($p < 0.01$, $F = 181$) effect of fluoxetine on MDMA-induced 5-HT release but not DA release

All animal procedures were approved by the Wayne State University Animal Investigation Committee and adhere to the NIH Guide for the Care and Use of Laboratory Animals.

Results

Microdialysis

The effects of (+)-MDMA (4.7 μmol/kg, i.v.) on striatal DA release in vivo in the presence and absence of the selective 5-HT uptake inhibitor fluoxetine

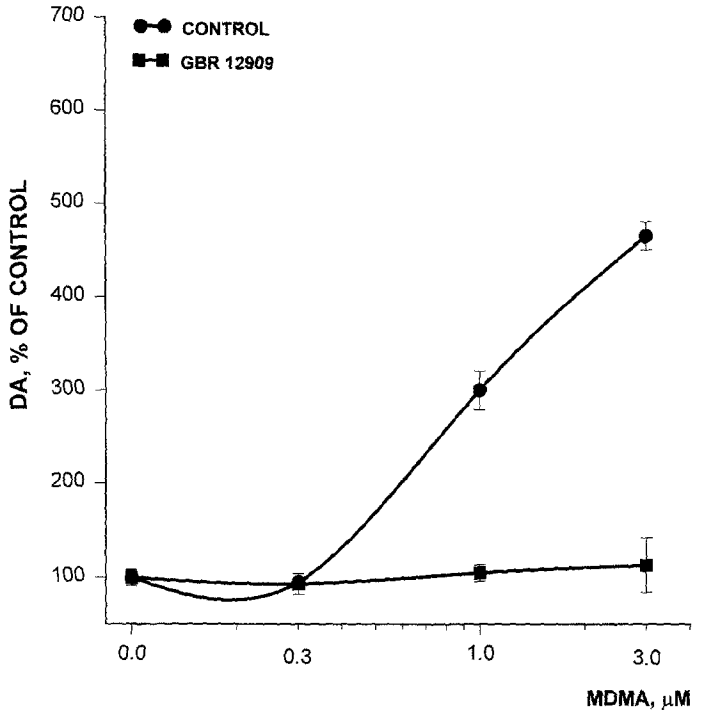

Fig. 3. The selective DA uptake inhibitor GBR 12909 (■, 1 μM) completely blocked MDMA-induced (0.3–3 μM DA release, suggesting that the effect of MDMA on DA in vitro is dependent on DA transporter function. Each data point represents the mean ± S.E.M. of n = 4 replicates. Two-way ANOVA revealed a significant ($p < 0.01$, $F = 120$) effect of GBR-12909 on MDMA-induced DA release

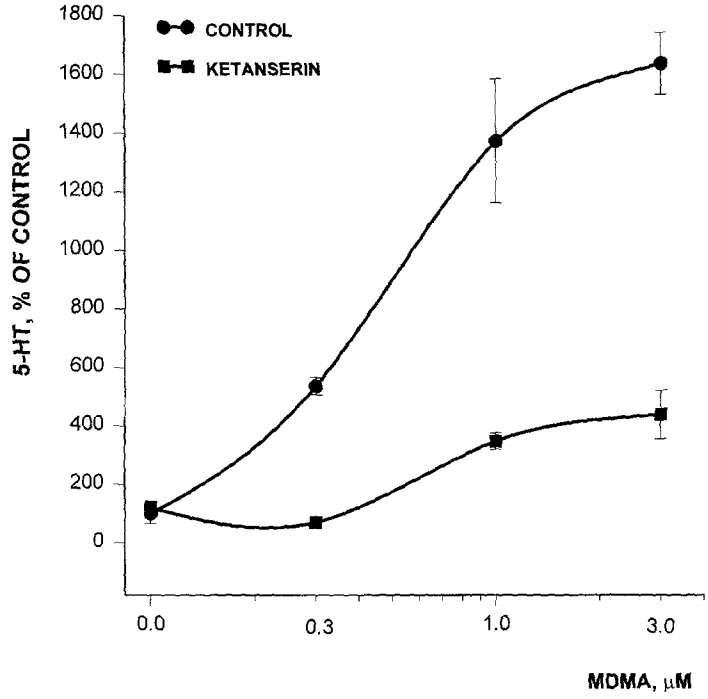

Fig. 4. The 5-HT_2 antagonist ketanserin (■, 1 μM) attenuated MDMA-induced (0.3–3 μM) 5-HT release, implicating an inhibitory effect of ketanserin on the 5-HT transporter. Each data point represents the mean ± S.E.M. of n = 4 replicates. Two-way ANOVA showed a significant ($p < 0.01$, $F = 108$) effect of ketanserin on MDMA-induced 5-HT release

(14.4 μmol/kg, s.c.) are presented in Fig. 1. Peripheral administration of (+)-MDMA significantly ($p < 0.01$, $n = 12$) increased extracellular levels of DA to 501 ± 66% of basal levels (Fig. 1). Fluoxetine administration alone had no significant effect on basal levels of extracellular striatal DA compared to control. However, after pretreatment with fluoxetine, the effect of (+)-MDMA on extracellular DA levels was significantly less than with (+)-MDMA alone (to 375 ± 48%, $p < 0.05$ compared to MDMA alone, $n = 8$).

Striatal slices

In vitro, (+)-MDMA (0.3–3 μM) increased extracellular levels of both, DA (Fig. 2) and 5-HT (Fig. 3) in a concentration-dependent manner, although the maximal effect on 5-HT release was more prominent. Fluoxetine (3 μM) completely blocked the ability of (+)-MDMA to increase extracellular 5-HT levels, but did not alter the effect of (+)-MDMA on DA levels.

Analysis of the data in Fig. 2 with a 2-way ANOVA revealed an overall significant ($p < 0.01$) effect of (+)-MDMA, fluoxetine and the interaction between (+)-MDMA and fluoxetine. Further analysis of each curve with 1-way ANOVA and Dunnett's t-test showed a significant effect of (+)-MDMA on 5-HT release in control, but not fluoxetine pretreated, slices. Analysis (2-way ANOVA) of the effect of (+)-MDMA on DA release (Fig. 3) revealed an overall significant effect of (+)-MDMA but not of fluoxetine or the interaction. Analysis of each curve with ANOVA and Dunnett's t-test showed a significant effect of (+)-MDMA on DA release in both control and fluoxetine pretreated slices. In contrast to the effect of fluoxetine, incubation with the selective DA uptake blocker GBR 12909 (1 μM) completely blocked (+)-MDMA's effect on DA release (Fig. 4), but not MDMA-induced 5-HT release (data not shown).

Since 5-HT_2 receptor antagonists prevent the serotonergic toxicity of (+)-MDMA in vivo, the potential effect of (+)-MDMA on the 5-HT transporter was determined in the presence of the 5-HT_2 antagonist ketanserin (1 μM). Under these conditions, ketanserin attenuated the ability of (+)-MDMA to increase 5-HT levels, but had no effect on DA levels (data not shown).

Discussion

Repeated exposure to MDMA [at doses corresponding to those ingested by humans, Ricaurte et al., (1988)] produces long-lasting, deleterious effects on the 5-HT system in several mammalian species, including rats and rhesus monkeys (see review by Mc Kenna and Peroutka, 1990). Although the potential toxicity of MDMA in humans remains to be demonstrated, the pernicious effect of MDMA on 5-HT neurons is of great concern considering the increasing popularity of MDMA as a recreational drug. To investigate further its underlying mechanism of action, (+)-MDMA was used in the present study as a probe to release endogenous 5-HT in vivo to study the influence of 5-HT on DA function. Since MDMA acts as a substrate for both DA and 5-HT transporters, selective inhibitors of monoamine transport were utilized in vivo and in vitro to dissect the effects of MDMA on each neuronal system.

Systemic administration of (+)-MDMA (4.7 μmol/kg, i.v.) increased extracellular levels of striatal DA approximately 5-fold (Fig. 1), in agreement with previously reported results (Yamamoto and Spanos, 1988; Nash, 1990; Gough et al., 1991; Nash and Yamamoto, 1992). This observation, combined with the fact that intrastriatal application of either 5HT or 5HT agonists increases extracellular striatal DA, (Benloucif and Galloway, 1991; Chen et al., 1992; Benloucif et al., 1993; Galloway et al., 1993; Parsons and Justice, 1993; Yadid et al., 1994; West and Galloway, 1996; Iyer and Bradberry, 1996), prompted us to investigate the potential role of endogenous 5HT in MDMA's effect on extracellular DA. When the effect of MDMA on 5HT release was blocked by pretreatment with fluoxetine (e.g., Bradberry, 1994), the ability of systemic MDMA to increase extracellular DA was significantly attenuated (Fig. 1). The concept that intact 5HT function is required for MDMA to enhance extracellular DA is consistent with Brodkin et al. (1993), who reported that acute depletion of 5-HT with p-chlorophenylalanine attenuated the increase of extracellular striatal DA produced by MDMA. Thus, the combined observations support the contention that in vivo, MDMA-induced 5-HT release contributes to the overall effect of MDMA on DA release.

In vitro, (+)-MDMA (0.3–3 μM) released both DA and 5-HT from striatal slices in a dose-dependent manner, although the effect was more efficacious for 5-HT (Figs. 2 and 3). Evidence that MDMA acts directly at both 5HT and DA transporters is presented in Figs. 2 and 3. Fluoxetine, a selective inhibitor of the 5HT transporter, completely inhibited the effect of (+)-MDMA on 5-HT release, but had no effect on extracellular DA. Similarly, GBR-12909, a selective inhibitor of the DA transporter, inhibited (+)-MDMA-induced DA release in vitro (Fig. 3), implying a significant role for the DA transporter in mediating this (+)-MDMA effect. Considering the above findings, it is reasonable to suggest that MDMA can release DA via two mechanisms in vivo: First, it can directly increase extracellular DA (presumably via the DA transporter) and second, MDMA can indirectly release DA via 5-HT facilitation (vide supra). The fact that 5-HT uptake blockers prevent both MDMA-induced release of 5-HT in vitro and 5-HT toxicity in vivo suggests an important role of the 5-HT transporter in mediating the acute and long-term effects of MDMA (Hetmatpanah and Peroutka, 1990; McCann and Ricaurte, 1993). The importance of the 5-HT transporter in the acute effect of MDMA has also been suggested by Schmidt and Taylor (1990), who found that inhibitors of the 5-HT transporter reversed the MDMA-induced decrease in tryptophan hydroxylase.

If elevated levels of extracellular 5HT influence DA release in situ, administration of antidepressants that are selective serotonin reuptake inhibitors (SSRIs) may ultimatley activate DA function, depending on either the extracellular 5-HT concentration or a particular DA projection field, such as the prefrontal cortex (Tanda et al., 1996). Although fluoxetine increases 5-HT levels in vivo (Rutter and Auerbach, 1993; Malagie et al., 1995), the differences in the mode of action between fluoxetine and MDMA may account for the absence of increased striatal DA levels after systemic fluoxetine administration (Fig. 1). Fluoxetine will transiently increase extracellular 5-HT with a

compensatory decrease in 5-HT unit activity (via stimulation of somatodendritic 5-HT autoreceptors) and, consequently, decreased impulse-dependent 5-HT release (Bel and Artigas, 1992; Galloway, 1996). Conversely, MDMA acting as a transporter substrate increases synaptic 5-HT irrespective of impulse flow and independent of autoreceptor influence. Thus, a threshold for facilitating DA release apparently exists between 5-HT levels achieved by the different treatment paradigms. Moreover, the effect of 5-HT on DA seems to be dependent on impulse flow in an intact system, since 5-HT facilitation of DA release was not evident in striatal slices, i.e., fluoxetine failed to affect (+)-MDMA-induced increases in DA release in vitro. The apparent necessity of an intact neuronal network points out a major advantage of in vivo microdialysis studies, as most brain functions are fully maintained under microdialysis conditions.

5-HT facilitation of DA release may relate to the potential role for DA in mediating MDMA-induced long-term deficits to the 5-HT neuronal system. It has been suggested that excessive extracellular DA, taken up into the 5-HT terminal, causes oxidative damage to the 5-HT neuron (Brodkin et al., 1992). The unexpected ability of ketanserin to block the effect of MDMA on 5-HT release in vitro (Fig. 4) may also be related to toxicity since pretreatment with ketanserin in vivo blocks MDMA-induced toxicity to 5-HT systems (Nash et al., 1990; Schmidt et al., 1990a). Since MDMA increases extracellular 5-HT levels through a transporter sensitive mechanism, it is plausible that ketanserin binds nonspecifically to the 5-HT transporter thus interfering with MDMA's ability to release 5-HT. Alternatively, 5-HT, acting through a 5-HT_2 receptor, may regulate the activity of the 5-HT transporter, analogous to the effect of DA on the DA transporter (Meiergerd et al., 1993; Parsons et al., 1993; Cass and Gerhardt, 1994).

Acknowledgements

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